

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/10	A1	(11) International Publication Number: WO 00/63362 (43) International Publication Date: 26 October 2000 (26.10.00)
(21) International Application Number: PCT/US00/10834 (22) International Filing Date: 21 April 2000 (21.04.00) (30) Priority Data: 60/130,353 21 April 1999 (21.04.99) US (71) Applicant: ANNOVIS, INC. [US/US]; 34 Mount Pleasant Drive, Aston, PA 19014 (US). (72) Inventors: MACCECCHINI, Maria-Luisa; 1223 Foxglove Lane, West Chester, PA 19388 (US). GORE, Mitchell, T.; 312 Gwynedd Court, Exton, PA 19341 (US). (74) Agents: PABST, Patrea, L. et al.; Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MAGNETIC DNA EXTRACTION KIT FOR PLANTS (57) Abstract A method and kit for the extraction of DNA from plants is provided, which quickly yields plant DNA with a high level of purity. The method isolates DNA (genomic, chloroplast, and/or mitochondrial DNA) using immobilized anionic groups, preferably on a chromatographic substrate or more preferably magnetic beads derivatized with anionic groups such as diethylaminoethyl (DEAE) via an anion-exchange interaction. The purified DNA is then eluted with ions (typically a salt solution). RNA can be removed by digestion with RNase.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

MAGNETIC DNA EXTRACTION KIT FOR PLANTS

Background of the Invention

The present invention generally relates to the field of methods and kits for the extraction of DNA and specifically to methods and kits for the extraction of DNA from plants.

Methods for extracting DNA from plants include:

The Dellaporta method. Dellaporta, S.L., et al. 1983. A plant DNA miniprep: Version II. *Plant Mol Biol Rep* 1:19-21. where DNA is precipitated to produce a very crude preparation, with a lot of the ethanol insoluble contaminants present in the solution. A variant of the Dellaporta method introduces an additional step of purifying the DNA via ultracentrifugation through a cesium chloride (CsCl) gradient for several hours or overnight. Richards, E., et al. 1994. *Current Protocols in Molecular Biology*, Suppl. 27, 2.3.1-2.3.7. Purifying DNA through CsCl gradients is an old established technique that can give very pure DNA, but is time consuming, expensive and results in very low yields.

An alternative DNA extraction method is the cetyltrimethylammonium bromide (CTAB) method. Murry, M.G. et al. 1980. *Nucleic Acids Res*, 8:4321-4325. CTAB is a cationic detergent, which will form an insoluble complex with the DNA in the presence of low concentrations of salt, such as a 0.5 M sodium chloride (NaCl) solution. In the CTAB method, the original lysis solution contains about 1.4 M NaCl. The DNA binds with the CTAB when the NaCl concentration is decreased to 0.5 M. This method is also time consuming and, because the procedure does not use organic solvents, an additional step needs to be included to clean up the DNA: a final extraction with organic solvents to rid the preparation of polysaccharide and phenolic compounds.

Methods for extraction of DNA from bacterial cells are more well established. Usually cells are lysed by placing the cells in an alkaline environment. Plasmid DNA is separated from bacterial chromosomal DNA by precipitating the chromosomal DNA and leaving the plasmid DNA in the

supernatant solution. As described in U.S. Patent No. 5,665,544 to Reeve, et al, after the chromosomal DNA is separated from the plasmid DNA, magnetic beads are introduced into the plasmid containing supernatant. The plasmid DNA binds to the beads, and the plasmid DNA is separated from the contaminants in the supernatant. U.S. Patent No. 4,935,342 to Seligson, et al., discloses a general method for the isolation and purification of nucleic acids, based on the binding to and elution from anion exchange columns, including the use of sequential columns containing weak and strong anion exchange materials. U.S. Patent No. 5,705,628 to Hawkins, discloses the use of magnetic microparticles with a coating including functional groups, specifically carboxyl or negatively charged groups, for the purification and isolation of DNA by binding and elution. International Application WO 96/18731 by Deggerdal, et al., discloses a method for the isolation of nucleic acids by the binding to and elution from a solid support, preferably magnetic beads, in the presence of a detergent, preferably an anionic detergent such as SDS or SARCOSYL™. U.S. Patent No. 5,650,506 to Woodard, et al., discloses the use of glass fiber membranes bearing positive surface charges for DNA purification by binding to and elution from the glass fibers.

A commercially available method for the extraction of DNA from bacterial cells is marketed as ISOLATE™ by Annovis, Inc. (catalog number 2-0300-85). The protocol includes the following steps: suspending bacterial cells in a buffer (pH 8.0), lysing the cells by mixing the suspension with an alkaline – detergent solution (0.2 M NaOH, 1% SDS), neutralizing the alkali and precipitating the chromosomal DNA with 3.0 M Potassium Acetate, pH 5.5, centrifuging the mixture to sediment the precipitated protein, cell debris and denatured chromosomal DNA, mixing the supernatant containing the plasmid DNA with a suspension containing magnetic beads, binding the DNA to the beads via an anionic interaction, eluting the DNA from the beads, and adjusting the salt concentration to 0.3, adding ethanol to render the plasmid DNA insoluble and effecting the precipitation of plasmid DNA from the liquid phase. This procedure does not work well with genomic plant DNA, however.

It is therefore an object of the present invention to provide a method of extracting and purifying DNA from plants with a high degree of purity and integrity in a short period of time.

It is also an object of the present invention to provide a kit with reagents for rapidly and inexpensively extracting and purifying DNA from plants.

Brief Summary of the Invention

A method and kit for the extraction of DNA from plants is provided, which quickly yields plant DNA with a high level of purity. The method isolates DNA (genomic, chloroplast, and/or mitochondrial DNA) using immobilized anionic groups, preferably on a chromatographic substrate or more preferably magnetic beads derivatized with anionic groups such as diethylaminoethyl (DEAE) via an anion-exchange interaction. The purified DNA is then eluted with ions (typically a salt solution). RNA can be removed by digestion with RNase.

Detailed Description of the Invention

The following terms are used herein:

anionic - positively charged

cationic - negatively charged

nonionic – non charged

Zwitterionic – same number of positive and negative charges on opposite ends so total compound is neutral.

The method described herein can be used with any plant material and has been demonstrated to be efficacious with the following representative types of plants: arabidopsis seedlings, barley embryos, tobacco leaves, tomato leaves, soybean hypocotylis and cultured cells, white beans hypocotylis and roots, young and old pine needles.

The process generally includes the steps of: grinding plant material to make a tissue extract, lysing the plant cells, removing cell debris, and binding the plant DNA to an immobilized or insoluble material such as magnetic beads, where it is separated into pure form.

In a preferred method, plant material is first ground to a powder in liquid nitrogen and then incubated in lysis buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.25 M NaCl and 100 microgram/ml proteinase K) in the presence of a surfactant or nonionic detergent such as N-laurylsarcosine (SARKOSYL™), TRITON™ or NONIDET™ P-40, which acts to solubilize cell components and lyse the cells. Representative detergents are listed in Table I.

Cell debris is then removed by centrifugation and the supernatant, which contains the DNA and other soluble cell components, is collected. The DNA is then bound to an immobilized or insoluble material having anionic groups bound thereto, such as magnetic beads derivatized with DEAE. This material is mixed with the supernatant, bound to the DNA, and then removed from the supernatant using a magnetic separator.

The beads are subsequently washed and then the DNA is preferably eluted from the beads by adding NaCl to a final concentration of 1.0 M. Alternatively, the DNA could be removed by binding to DEAE chromatographic material or filler material, which is separated by washing, centrifugation, or other methods known to those skilled in the art.

The method and kit produces DNA of equal purity to CsCl gradient methods at yields that are equal to, or better than, the prior art, Dellaporta and CTAB methods. The specific problems of the Dellaporta method (use of a CsCl column), and CTAB method (use of cationic detergents), are avoided by this method. The method and kit also extracts and purifies the DNA more quickly than the CsCl method. The method including the precipitation step takes a total of approximately 2.5 hours; without the precipitation step it takes less than 2 hours (1 hour and 50 minutes) to extract and purify the plant DNA: approximately 1 hour to lyse the cells, 5 minutes to bind the DNA to the magnetic beads, 5 minutes to wash the beads, 5 minutes to separate the DNA from the beads, 30 minutes to precipitate the DNA from solution, and 40 minutes to collect the DNA by centrifugation. In contrast, the CsCl centrifugation requires many hours to purify the DNA.

The preferred method described herein produces a very pure DNA preparation via an anion-exchange interaction where DNA is replaced as the binding species on the anion-exchange matrix by chloride ions or other negatively charged ions derived from any of a variety of salts. If a traditional anion-exchange column were used at the point where the beads are introduced, the columns would likely clog, preventing collection of bound DNA, or if the elution was effected with strong acid or base, significant levels of contaminants would co-elute with the DNA. In contrast, since the DNA bound to the beads can be thoroughly mixed with the wash solutions, contaminants are more easily removed than they are in traditional column formats, resulting in a more pure preparation. Moreover, because the DNA bound to the beads is not sheared or compressed, when pelleted, the attached DNA consists of longer and more intact strands.

The lysis methods for plant genomic DNA and bacterial plasmid DNA are different. The lysis method for bacterial plasmid DNA uses alkaline conditions in the presence of anionic detergent in the form of SDS, while the lysis method for plant genomic DNA uses a nonionic detergent. The type of detergent used affects the remaining steps in each method. Since SDS is anionic (negatively charged, like DNA), it must be removed from the solution in the plasmid procedure before the beads are introduced, otherwise the SDS would compete for binding with the plasmid DNA on the positively charged beads. In the ISOLATE™ plasmid extraction system, the SDS is removed from the solution by adding potassium acetate to form an insoluble precipitate with the chromosomal DNA. The aggregate of SDS with bacterial genomic DNA can be easily separated from the soluble plasmid DNA. In contrast, in the plant system, no precipitation is needed prior to the binding step, because the nonionic non-charged detergent used in the methods and kits described herein does not compete with the DNA for binding sites, since the genomic DNA is the desired binding species for the DEAE groups on the beads. Therefore, the nonionic detergent does not need to be removed, whereas SDS and other anionic detergents are negatively charged

and do compete with DNA for anionic binding sites and therefore would need to be removed.

Example 1: Purification of Plant Genomic DNA.

The following steps describe extraction and purification of DNA from plants.

1. Harvest approximately 0.5 grams fresh plant tissue. Rinse tissue with deionized (d.I). water to remove adhering debris and blot dry.
2. Freeze tissue with liquid nitrogen and grind to a fine powder in a mortar and pestle. Transfer to a 30 mL conical tube. Alternatively, fresh tissue may be mechanically homogenized in cold lysis buffer at 4°C.
3. Add 5-20 ml of lysis buffer/gram of starting material (0.5 to 0.01 M Tris-HCl, pH 7.0 - 8.0, 0.001 - 0.5 M EDTA, pH 7.0 - 8.0, 0.05 - 0.4 M NaCl and 50 - 500 µg/ml proteinase K) to the frozen powder and mix well.
4. Add N-lauroylsarcosine at a final concentration of between 0.1% and 10%. If desired, 25 - 200 µg of RNase A can be added at this point. Alternatively, the final resuspended pellet can be treated with RNase. Incubate 30 minutes to 1 hour at 50-60°C.
5. Centrifuge lysate 10 min at greater than 5000x g at 4°C to pellet debris. Remove the supernatant to a fresh tube and filter, if necessary, to remove undigested debris.
6. Add from 1 - 30 mL of magnetic beads (40 % v/v suspension) to supernatant. Gently mix the bead-supernatant suspension by inversion or mechanical mixer at room temperature for at least 1 minute.
7. Immobilize the magnetic beads in the magnetic separator and remove supernatant.
8. Add between 5 and 20 ml of wash buffer, cap and remove the tube from the stand and gently mix for 5-10 minutes at room temperature.
9. Immobilize the magnetic beads in the magnetic separator and remove supernatant. Repeat wash. if supernatant is substantially pigmented.

10. Add between 5 and 10 ml of elution buffer (0.7 - 1.5 M NaCl, 0.5 to 0.01 M Tris-HCl, pH 7.0 - 8.0, 0.001-0.5 M EDTA, pH 7.0 - 8.0) to the magnetic beads and mix for 5 - 10 minutes at room temperature.
11. Immobilize the magnetic beads in the magnetic separator and transfer supernatant to a fresh tube.
12. Add 1.0 to 2.5 volumes of cold isopropanol. Mix well and incubate at -20°C for at least 10 min.
13. Collect DNA by centrifugation at 10,000 - 20,000 x g for at least 15 min at 4°C. Wash pellet with 70% cold ethanol and re-centrifuge at 10,000 - 20,000 x g for at least 2 min at 4°C. Allow the pellet to air-dry at room temperature and then dissolve in desired buffer.

It is understood that the disclosed method and kit is not limited to the particular methodology, protocols, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

**Table I: Representative Detergents which can be used in the Plant
DNA Extraction Method** (available from Sigma Chemical Co)

Anionic Detergents

Aerosol 22	Glycodeoxycholic Acid
Aerosol®-OT	1-Heptanesulfonic Acid
Salts of:	1-Hexanesulfonic Acid
Alginic Acid	N-Lauroylsarcosine
Caprylic Acid	Lauryl Sulfate (Dodecyl sulfate)
Cholic Acid	1-Nonanesulfonic Acid
1-Decanesulfonic Acid	1-Octanesulfonic Acid
Dehydrocholic Acid	1-Pentanesulfonic Acid
Deoxycholic Acid	Taurocholic Acid
Diethyl Sulfosuccinate	Taurodeoxycholic Acid
1-Dodecanesulfonic Acid	Niaproof (formerly Tergitol)
Glycocholic Acid	

Cationic Detergents

Alkyltrimethylammonium Bromides
Benzalkonium Chloride
Benzothonium Chloride
Benzyltrimethylammonium Bromide
Benzyltrimethylhexadecylammonium Chloride
Benzyltrimethyletetradecylammonium Chloride
Cetyltrimethylethylammonium Bromide
Cetylpyridinium
Decamethonium Bromide
Dimethyldioctadecylammonium Bromide
Methylbenzethonium Chloride
Methyltriethylammonium Chloride
N, N', N'-Polyoxyethylene(10)-N-tallow-1, 3-diaminopropane

Zwitterionic Detergents

CHAPS
CHAPSO
N-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
N-Dodecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate
N-Hexadecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate
N-Octadecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate
N-Octyl-N, N-dimethyl-3-ammonio-1-propanesulfonate
Phosphatidylcholine
N-tetradecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate

Table I continued.**Nonionic Detergents**

BIGCHAP
Decanoyl-N-methylglucamide
n-Decyl -D-Glucopyranoside
n-Decyl -D-Glucopyranoside
n-Decyl -D-Maltopyranoside
Deoxy-BIGCHAP
n-Dodecyl -D-Glucopyranoside
n-Dodecyl -D-Maltoside
n-Dodecyl -D-Maltoside
Heptanoyl-N-methylglucamide
n-Heptyl -D-Glucopyranoside
N-Heptyl -Thioglucopyranoside
n-Hexyl -D Glucopyranoside
Igepal CA-630
1-Monooleoyl-rac-glycerol
Nonanoyl-N-methylglucamide

n-Nonyl -D-Glucopyranoside
n-Nonyl -D-Glucopyranoside
Octanoyl-N-methylglucamide
n-Octyl -D-Glucopyranoside
n-Octyl -D-Glucopyranoside
Octyl -D-Thiogalactopyranoside
Polyoxyethylene Esters
Polyoxyethylene Ethers
Polyoxyethylenesorbitan Esters
Sorbitan Esters
Tergitol
n-Tetradecyl -D-Maltoside
Tritons
Tyloxapol
n-Undecyl -D-Glucopyranoside

We claim:

1. A method for extracting DNA from plant material comprising
 - (a) lysing the cells in the plant material,
 - (b) removing the plant cell material debris,
 - (c) binding the DNA to positively charged groups which are immobilized or insoluble,
 - (d) separating the bound DNA from the remaining cellular material, and
 - (e) separating the bound DNA from the positively charged groups.
2. The method of claim 1 wherein a nonionic detergent is used to lyse the cells.
3. The method of claim 1 wherein the positively charged groups are DEAE.
4. The method of claim 1 wherein the positively charged groups are on magnetic beads.
5. The method of claim 1 wherein the plant cell debris is removed by centrifugation.
6. The method of claim 1 wherein the bound DNA is separated by increasing salt ion concentration.
7. The method of claim 1 further comprising adding RNA to digest the RNA in the plant material or purified DNA.
8. A kit for use in a method for extracting DNA from plant material comprising
 - (a) a solution for lysing the cells in the plant material,
 - (b) positively charged groups which are immobilized or insoluble, and
 - (c) a salt solution for separating the bound DNA from the positively charged groups.
9. The kit of claim 8 wherein the solution to lyse the cells is a non-ionic solution.

10. The kit of claim 8 wherein the positively charged groups are DEAE immobilized on a chromatographic substrate or magnetic beads.

11. The kit of claim 8 comprising:

- (a) lysis buffer comprising 0.5 to 0.01 M Tris-HCl, pH 7.0 - 8.0, 0.001 - 0.5 M EDTA, pH 7.0 - 8.0, 0.05 - 0.4 M NaCl and 50 - 500 $\mu\text{g/ml}$ proteinase K);
- (b) N-lauroylsarcosine;
- (c) magnetic beads having DEAE groups on the surface thereof; and
- (d) elution buffer comprising 0.7 - 1.5 M NaCl, 0.5 to 0.01 M Tris-HCl, pH 7.0 - 8.0, 0.001-0.5 M EDTA, pH 7.0 - 8.0.

12. The kit of claim 8 further comprising RNA.

13. The kit of claim 11 further comprising RNA.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/10834

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 91 07422 A (BOEHRINGER MANNHEIM CORP) 30 May 1991 (1991-05-30) page 6, line 29 - line 31 page 7, line 12 -page 9, line 20 page 10, line 7 - line 36 page 17, line 10 - line 15 abstract ----	1-3, 5-10, 12 3, 4, 10, 11, 13
X Y	WO 96 18731 A (DZIEGLEWSKA HANNA EVA ; DYNAL AS (NO); LARSEN FRANK (NO); DEGGERDAL) 20 June 1996 (1996-06-20) page 5, line 30 -page 6, line 11 page 7, line 2 - line 15 page 7, line 28 -page 8, line 1 page 11, line 3 - line 12 ----- -/--	1, 2, 4-9, 12 1-13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 August 2000

Date of mailing of the international search report

21/08/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Oderwald, H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/10834

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 04730 A (PROMEGA CORP) 5 February 1998 (1998-02-05) page 11, line 28 -page 13, line 10 page 15, line 25 -page 16, line 9 page 16, line 22 -page 17, line 4 ----	1-13
P,X	WO 99 29703 A (BAKER MATTHEW JOHN ;DNA RESEARCH INSTR LIMITED (GB)) 17 June 1999 (1999-06-17) page 2, line 10 - line 18 page 3, line 27 - line 30 -----	1,2,4,5, 7

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/10834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9107422 A	30-05-1991	US 4997932 A AT 151433 T DE 69030449 D DE 69030449 T DE 69033304 D DE 69033304 T EP 0508985 A EP 0747387 A EP 0747388 A ES 2100887 T ES 2139292 T	05-03-1991 15-04-1997 15-05-1997 16-10-1997 28-10-1999 03-02-2000 21-10-1992 11-12-1996 11-12-1996 01-07-1997 01-02-2000
WO 9618731 A	20-06-1996	AU 706211 B AU 4182996 A CA 2207608 A EP 0796327 A JP 11501504 T	10-06-1999 03-07-1996 20-06-1996 24-09-1997 09-02-1999
WO 9804730 A	05-02-1998	US 5981235 A AU 3821497 A EP 0918877 A	09-11-1999 20-02-1998 02-06-1999
WO 9929703 A	17-06-1999	AU 1344799 A	28-06-1999